# This Page Is Inserted by IFW Operations and is not a part of the Official Record

# **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

# IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents will not correct images, please do not report the images to the Image Problem Mailbox.

THIS PAGE BLANK (USPTO)

# **PCT**

# WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5:
C12N 9/02, D21C 9/10, C11D 3/386

(11) International Publication Number: WO 95/01426
(43) International Publication Date: 12 January 1995 (12.01.95)

(21) International Application Number: PCT/DK94/00210 (81) Designated States: AU, BB, BG, BR, BY, CA, CI HU, JP, KP, KR, KZ, LK, LV, MG, MN, MW (22) International Filing Date: 31 May 1994 (31.05.94) PL, RO, RU, SD, SK, UA, US, UZ, VN, Europ

(30) Priority Data:
773/93 29 June 1993 (29.06.93) DK

(71) Applicant (for all designated States except US): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsvaerd (DK).

(72) Inventors; and
(75) Inventors/Applicants (for US only): SCHNEIDER, Palle [DK/DK]; Rydtoften 43, DK-2750 Ballerup (DK). PED-ERSEN, Anders, Hjelholt [DK/DK]; Nybro Vænge 58, DK-2800 Lyngby (DK).

(74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsvaerd (DK).

(81) Designated States: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

#### (54) Title: ENHANCEMENT OF LACCASE REACTIONS

#### (57) Abstract

This invention relates to methods of oxidizing a substrate in the presence of a laccase or a laccase related enzyme and an enhancing agent. More specifically, the invention relates to a method of bleaching of dye in solutions, to a method of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, to a method of bleaching of lignin-containing material, in particular bleaching of pulp for paper production, to a method of treatment of waste water from pulp manufacturing, and to a method of enzymatic polymerization and/or modification of lignin containing material.

## FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
ΑÜ	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	BU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	TT.	Italy	PL	Poland
-			-	PT	Portugal
BR	Brazil	JP	Japan	RO	Romania
BY	Belarus	KE	Кепуа	_	Russian Federation
CA	Canada	KG	Kyrgystan	RU	
CF	Central African Republic	KP	Democratic People's Republic	SD	Sudan
CG	Congo		of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	£.K	Sri Lanka	TD	Chad
cs	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
· DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	MIL	Mali	UZ	Uzhekistan
FR	France	MIN	Mongolia	VN	Vict Nam
GA	Gabon		· · · · · · · · · · · · · · · · · · ·		

WO 95/01426 PCT/DK94/00210

## ENHANCEMENT OF LACCASE REACTIONS

#### TECHNICAL FIELD

This invention relates to activation of enzymes.

More specifically, the invention relates to agents capable of senhancing the activity of laccases and related enzymes.

The invention also relates to methods of oxidizing a substrate in the presence of a laccase enzyme, and an enhancing agent. More specifically, the invention relates to a method of bleaching of dye in solutions, to a method of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, to a method of bleaching lignin-containing material, in particular bleaching of pulp for paper production, to a method of treatment of waste water from pulp manufacturing, and to a method of enzymatic polymerization and/or modification of lignin or lignin containing material.

#### BACKGROUND ART

Laccases (E.C. 1.10.3.2) are enzymes that catalyse the oxidation of a substrate (an electron or hydrogen donor) with oxygen. Such enzymes are known from microbial, plant and animal origins, e.g. from fungi. They are typically copper proteins, i.e. they contain a copper atom or atoms as a prosthetic group.

Use of laccases has been suggested e.g. in bleaching 25 of pulp for paper production, in treatment of waste water from pulp production, for improved bleaching in laundry detergents, for dye transfer inhibition during laundering, and for lignin modification, e.g. in particle board production.

The compound 2,2'-azino-bis(3-ethylbenzothiazoline-30 6-sulfonate), ABTS, supplied by Boehringer Mannheim, is a chromogenic substrate, and a common peroxidase and phenol oxidase assay agent. These enzymes catalyse the oxidation of ABTS by hydrogen peroxide and dioxygen, respectively, producing a greenish-blue colour, which process may be monitored photometrically.

ABTS has been found to form a stable radical cation when oxidized by a laccase enzyme (polyphenol oxidase, EC 1.10.3.2), and has been proposed to act as a redox mediator for oxidation of non-phenolic lignin model compounds [Bourbonnais R, Paice M G; FEBS Lett (1990) 267 99-102].

Studies on demethylation and delignification of 10 kraft pulp by a laccase enzyme in the presence of ABTS showed that the extent of partial demethylation by laccase was increased in the presence of ABTS [Bourbonnais, R. and Paice, M.G; Appl. Microbiol. Biotechnol. (1992) 36 823-827].

Certain oxidizable substrates e.g. metal ions and 15 phenolic compounds such as 7-hydroxycoumarin (7HCm), vanillin (VAN), and p-hydroxybenzenesulfonate (pHBS), have been described as accelerators or enhancers, able to enhance bleaching reactions (cf. e.g. WO 92/18683, WO 92/18687, and Kato M and Shimizu S, Plant Cell Physiol. 1985 26 (7), pp. 1291-1301 (cf. Table 1 in particular), or Saunders B C, et al., Peroxidase, London, 1964, p. 141 ff).

#### SUMMARY OF THE INVENTION

It has now surprisingly been found that organic chemical compounds consisting of at least two aromatic rings, 25 of which aromatic rings at least one ring is substituted with one or more of the following atoms: nitrogen, oxygen, and sulfur, and which aromatic rings may furthermore be fused rings, are capable of enhancing the activity of laccase enzymes.

Accordingly, in its first aspect, the invention provides an agent for enhancing the activity of laccase enzymes, which agent is an organic chemical compound consisting of at least two aromatic rings, of which aromatic rings at least one ring is substituted with one or more of the following

.

atoms: nitrogen, oxygen, and sulfur; and which aromatic rings may furthermore be fused rings.

In a more specific aspect, the invention provides an agent for enhancing the activity of laccase enzymes, which sagent is an organic chemical compound of the general formula I:

in which general formula A represents a single bond, or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-CR^{11}=N-N=CR^{15}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{12}=N-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-)_n$ ,  $(-CR^{12}=N-NR^{11}-)$ ,  $(-NR^{11}-CR^{12}=CR^{13}-)$ , (-N=N-), in which groups n represents an integer of from 1 to 6; or A represents carbon, carbonyl, nitrogen, sulfur, oxygen, selenium, or phosphor, which carbon, phosphor and nitrogen may be unsubstituted or substituted with a substituent group  $R^{11}$ ;

and in which general formula the substituent groups R1-R13 and R15, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy, sulfandiyl, nitro, amino, phenyl, C1-20 C14-alkyl, C1-C5-alkoxy, carbonyl-C1-C5-alkyl, aryl-C1-C5-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R14; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R14; and which 25 C1-C14-alkyl, C1-C5-alkoxy, carbonyl-C1-C5-alkyl, and aryl-C1-C5-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R14;

which substituent group  $R^{14}$  represents any of the 30 following radicals: hydroxy, formyl, carboxy and esters and

4

salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula R<sup>5</sup> and R<sup>6</sup> may together form a group -B-, in which B represents a single bond, the group (-CH=CH-)<sub>n</sub>, or the group (-CH=N-)<sub>n</sub>, in which groups n represents an integer of from 1 to 6; or B represents carbon, 20 nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group R<sup>14</sup> as defined above;

or in which general formula two of the substituent groups  $R^{1}-R^{10}$  may together form a group -C-, in which C respresents any of the following groups:  $(-CHR^{11}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ , in which groups n represents an integer of from 2 to 4, and in which groups  $R^{11}$  is a substituent group as defined above;

or in which general formula  $R^5$  and  $R^{12}$ , and/or  $R^6$  and  $R^{13}$ , when n in the above formula being 1, may together form a group -D-, in which D represents the groups: (-CHR<sup>11</sup>-), (-NR<sup>11</sup>-), (-CR<sup>11</sup>=CR<sup>15</sup>-), (-CR<sup>11</sup>=N-), (-N=CR<sup>11</sup>-), (-O-), (>C=O) or (-S-), and in which groups  $R^{11}$  and  $R^{15}$  are substituent groups as defined above.

In another specific aspect, the invention provides an agent for enhancing the activity of a laccase enzyme, which

agent is an organic chemical compound of the general formula II:

in which the substituent groups R<sup>1</sup>-R<sup>8</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>9</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>;

which substituent group R<sup>9</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups  $R^1-R^8$  may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-),  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n represents an integer of from 1 to 3,  $R^9$  is a substituent group as defined above and  $R^{10}$  is defined as  $R^9$ .

In another aspect, the invention provides a method for oxidizing a substrate with a laccase or a laccase related 15 enzyme, in the presence of oxygen and in the presence of an enhancing agent of the invention.

In a more specific aspect, the invention provides a method for bleaching dye in solutions by treatment with a laccase or a laccase related enzyme in the presence of oxygen 20 in the presence of an enhancing agent of the invention.

In another specific aspect, the invention provides a method of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the method comprising treatment of the wash liquor with a laccase or a laccase related enzyme in the presence of oxygen and in the presence of an enhancing agent of the invention.

In another aspect, the invention provides a method of bleaching lignin-containing material, in particular bleaching of pulp for paper production, the method comprising treatment of the lignin or lignin containing material with a laccase or a laccase related enzyme in the presence of a source of oxygen and in the presence of an enhancing agent of the invention.

In a further aspect, the invention provides a method of enzymatic polymerization and/or modification of lignin or

lignin containing material, the method comprising treatment of the lignin or lignin containing material with a laccase or a laccase related enzyme in the presence of a source of oxygen and in the presence of an enhancing agent of the invention.

In a yet further aspect, the invention provides a method of treatment of waste water, in particular waste water from pharmaceutical or chemical industry, e.g. waste water from dye manufacturing, from textile industry, or from pulp manufacturing, the method comprising treatment of the waste water with a laccase or a laccase related enzyme in the presence of a source of oxygen and in the presence of an enhancing agent of the invention.

In a particular aspect, the invention provides a detergent additive capable of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the detergent additive comprising an enzyme exhibiting laccase activity and oxygen and an enhancing agent of the invention.

In other aspects, the invention provides detergent additives and detergent compositions capable of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the detergent composition comprising an enzyme exhibiting laccase activity and oxygen and an enhancing agent of the invention.

#### BRIEF DESCRIPTION OF DRAWINGS

25

The present invention is further illustrated by reference to the accompanying drawings, in which:

Fig. 1 shows the specific formulas of some enhancing agents of the invention (I) 6-hydroxy-2-naphtoic acid; (II) 7-30 methoxy-2-naphtol; (III) 7-amino-2-naphthalene sulfonic acid; (IV) 5-amino-2-naphthalene sulfonic acid; (V) 1,5-diamino-naphthalene; (VI) 7-hydroxy-1,2-naphthimidazole; (VII) 10-methylphenothiazine; (VIII) 10-phenothiazine-propionic acid; (IX) N-hydroxysuccinimide-10-phenothiazine-propionate; (X)

benzidine; (XI) 3,3'-dimethylbenzidine; (XII) 3,3'-dimethoxybenzidine; (XIII) 3,3',5,5'-tetramethylbenzidine; (XIV) hydroxy-4-biphenylcarboxylic acid; (XV) 4-amino-4'-methoxystilbene; (XVI) 4,4'-diaminostilbene-2,2'-disulfonic acid; 5 (XVII) 4,4'-diaminodiphenylamine; (XVIII) 2,7-diaminofluorene; (XIX) 4,4'-dihydroxy-biphenylene; (XX) triphenylamine; (XXI) 10-ethyl-4-phenothiazinecarboxylic acid; (XXII) 10-ethyl-(XXIII) 10-propylphenothiazine; (XXIV) phenothiazine; isopropylphenothiazine; (XXV) methyl-10-phenothiazinepropion-10-phenylphenothiazine; (XXVII) 10-allyl-(XXVI) phenothiazine; (XXVIII) 10-phenoxazinepropionic acid; (XXIX) 10-(3-(4-methyl-1-piperazinyl)propyl)phenothiazine; (XXX) 10-(2-pyrrolidinoethyl)phenothiazine; (XXXI) 10-methylphenoxazine; (XXXII) iminostilbene; (XXXIII) 2-(p-aminophenyl)-6-methylben-15 zothiazole-7-sulfonic acid; (XXXIV) N-benzylidene-4-biphenylamine; (XXXV) 5-amino-2-naphthalenesulfonic acid; (XXXVI) 7-4,4'-dihydroxybenzophenone; methoxy-2-naphtol; (XXXVII) (XXXVIII) N-(4-(dimethylamino)benzylidene)-p-anisidine; (XXXIX) 3-methyl-2-benzothiazolinone(4-(dimethylamino)benzylidene)hy-20 drazone; (XL) 2-acethyl-10-methylphenothiazine; (XLI) 10-(2hydroxyethyl)phenothiazine; (XLII) 10-(2-hydroxyethyl)phenoxazine; (XLIII) 10-(3-hydroxypropyl)phenothiazine; (XLIV) 4,4'dimethoxy-N-methyl-diphenylamine; (XLV) vanillin azine.

Fig. 2 shows the bleaching of Direct Blue 1 with 25 bilirubin oxidase and 10-methylphenothiazine (MPT) (  $\square$  0  $\mu$ M MPT;  $\blacksquare$  5.0  $\mu$ M MPT;  $\lozenge$  10.0  $\mu$ M MPT).

#### DETAILED DISCLOSURE OF THE INVENTION

#### The Enhancing Agent

The present invention relates to the use of chemical compounds for enhancing the activity of laccase enzymes. Accordingly, the invention provides an agent capable of enhancing the effect of a laccase enzyme in the following termed enhancing agent.

9

The enhancing agent of the invention is an organic chemical compound consisting of at least two aromatic rings, of which aromatic rings at least one ring is substituted with one or more nitrogen, oxygen, and/or sulfur atoms, and which aromatic rings may furthermore be fused rings.

In a more preferred embodiment, the enhancing agent of the invention is an organic chemical compound of the general formula I:

in which general formula A represents a single bond, or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-CR^{11}=N-N=CR^{15}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{12}=N-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-)_n$ ,  $(-CR^{12}=N-NR^{11}-)$ ,  $(-NR^{11}-CR^{12}=CR^{13}-)$ , (-N=N-), in which groups n represents an integer of from 1 to 6; or A represents carbon, carbonyl, nitrogen, sulfur, oxygen, selenium, or phosphor, which carbon, phosphor and nitrogen may be unsubstituted or substituted with a substituent group  $R^{11}$ ;

and in which general formula the substituent groups R<sup>1</sup>-R<sup>13</sup> and R<sup>15</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy, sulfandiyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>1</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>14</sup>; and which phenyl may furthermore be unsubstituted or

substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1-C_1-alkyl$ ,  $C_1-C_5-alkoxy$ , carbonyl- $C_1-C_5-alkyl$ , and  $aryl-C_1-C_5-alkyl$  groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

which substituent group R14 represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, aminoalkyl, piperidino, amino, phenyl, sulfamoyl, nitro, 10 piperazinyl, pyrrolidino, C,-C,-alkyl,  $C_1-C_5-alkoxy;$ carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>5</sub>alkyl, C,-C5-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: 15 hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the 20 following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula R<sup>5</sup> and R<sup>6</sup> may together form a group -B-, in which B represents a single bond, the 25 group (-CH=CH-)<sub>n</sub>, or the group (-CH=N-)<sub>n</sub>, in which groups n represents an integer of from 1 to 6; or B represents carbon, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group R<sup>14</sup> as defined above;

or in which general formula two of the substituent groups  $R^1-R^{10}$  may together form a group -C-, in which C represents any of the following groups:  $(-CHR^{11}-N=N-)$ , (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>, in which groups n represents an integer of from 2 to 4, and in which groups  $R^{11}$  is a substituent group as defined 35 above;

or in which general formula  $R^5$  and  $R^{12}$ , and/or  $R^6$  and  $R^{13}$ , when n in the above formula being 1, may together form a group -D-, in which D represents the groups:  $(-CHR^{11}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{11}=CR^{15}-)$ ,  $(-CR^{11}=N-)$ ,  $(-N=CR^{11}-)$ , (-O-), (>C=O) or (-S-), and 5 in which groups  $R^{11}$  and  $R^{15}$  are substituent groups as defined above.

In particular embodiments, the enhancing agent is 2-(p-aminophenyl)-6-methylbenzothiazole-7-sulfonic acid, N-(4-(dimethylamino)benzylidene)-p-anisidine, 3-methyl-2-benzothiazolinone(4-(dimethylamino)benzylidene)hydrazone, vanillin azine.

In another preferred embodiment, the enhancing agent of the invention is an organic chemical compound of the general formula II:

in which the substituent groups R<sup>1</sup>-R<sup>8</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>9</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-

alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ;

which substituent group R9 represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be 10 unsubstituted or substituted once or twice with hydroxy,  $C_1 - C_3$ alkyl, C,-C,-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; 15 and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, 20 and sulfamovl;

or in which general formula two of the substituent groups  $R^1-R^8$  may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>,  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n represents an integer of from 1 to 3,  $R^9$  is a substituent group as defined above and  $R^{10}$  is defined as  $R^9$ .

In a more specific embodiment, the enhancing agent of the invention is an organic chemical compound of the following formula:

in which formula E represents a single bond, a carbonyl group or one of the following groups:  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or  $(-NR^{11}-)$ , in which n represents an integer from 1 to 2. The substituents groups  $R^1-R^{11}$  may be identical or different, 5 independently being one of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl,  $aryl-C_1-C_5-alkyl;$  which carbamoyl, sulfamoyl, and amino groups 10 may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ alkyl, and  $aryl-C_1-C_5$ -alkyl groups may be saturated or un-15 saturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R14;

which substituent group R14 represents any of the following radicals: hydroxy, formyl, carboxy and esters and 20 salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1 - C_3$ alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be 25 substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may 30 furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which specific formula two of the substituent 35 groups  $R^5$  and  $R^6$  may together form a group -B-, in which B represents the groups:  $(-CH=N-)_n$ , (-CH=CH-) or  $(-CHR^{14}-)$  in which

groups n represents an integer of from 1 to 2 and  $R^{14}$  is a substituent group as defined above.

In particular embodiments, the enhancing agent is 4-amino-4'-methoxystilbene, 4,4'-diaminostilbene-2,2'-disulfonic acid, iminostilbene, 4,4'-dihydroxybenzophenone, N-benzylidene-4-biphenylamine, 4,4'-diaminodiphenylamine, 4,4'-dimethoxy-N-methyl-diphenylamine, 2,7-diaminofluorene, triphenylamine.

In another specific embodiment, the enhancing agent may be described by the following formula:

in which formula X represents one of the following groups: (-O-), (-S-),  $(-NR^{15}-)$ ,  $(-CHR^{15}-)$ , (>C=O), (-CH=CH-), (-CH=CH-)CH=N-) and the substituent groups  $R^1-R^9$  and  $R^{15}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and 15 esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C,-C14-alkyl, C1-C5alkoxy, carbonyl-C,-C,-alkyl, aryl-C,-C,-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R10; and 20 which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R10; and which C1-C14-alkyl, C,-C5-alkoxy, carbonyl-C,-C5-alkyl, and aryl-C1-C5-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or 25 more substituent groups R<sup>10</sup>;

which substituent group  $R^{10}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino,

piperazinyl, pyrrolidino, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent 15 groups  $R^1-R^8$  may together form a group -B-, in which B represents any of the following the groups:  $(-CHR^{10}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or  $(-N=CR^{10}-NR^{11}-)$ , in which groups n represents an integer of from 1 to 3,  $R^{10}$  is a substituent group as defined above and  $R^{11}$  is defined as  $R^{10}$ .

In particular embodiments, the enhancing agent is 10-methylphenothiazine, 10-phenothiazine-propionic acid, Nhydroxysuccinimide-10-phenothiazine-propionate or 10-ethyl-4phenothiazine-carboxylic acid, 10-ethylphenothiazine, methyl-10propylphenothiazine, 10-isopropylphenothiazine, 25 phenothiazinepropionate, 10-phenylphenothiazine, 10-allylphenothiazine, 10-(3-(4-methyl-1-piperazinyl)propyl)phenothiazine, 2-acetyl-10-methyl-10-(2-pyrrolidinoethyl)phenothiazine, 4-carboxy-10-phenothiazine, phenothiazine, 10-phenoxazine-propionic phenoxazine, 10-ethylphenoxazine, 30 acid, 4-carboxy-10-phenoxazine-propionic acid, 10-(2-hydroxyethyl)phenothiazine, 10-(2-hydroxyethyl)phenoxazine or 10-(3hydroxypropyl) phenothiazine.

In another specific embodiment, enhancing agent is a biphenyl derivative of the following formula:

16

in which the substituent groups R<sup>1</sup>-R<sup>10</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>11</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>11</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>11</sup>;

which substituent group R11 represents any of the 15 following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be 20 unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; 25 and which  $C_1 - C_5$ -alkyl, and  $C_1 - C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, 30 and sulfamoyl.

In particular embodiments, the enhancing agent is benzidine, 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine,

3,3',5,5'-tetramethylbenzidine, 4'-hydroxy-4-biphenylcarboxylic acid, or 4,4'-dihydroxybiphenylene.

In another specific embodiment, the enhancing agent is a naphthalene derivative of the following formula:

in which the substituent groups R<sup>1</sup>-R<sup>8</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-10 alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>9</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-15 alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>;

which substituent group  $R^9$  represents any of the 20 following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be

unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_3$ -alkyl,  $C_1$ - $C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups  $R^1-R^8$  may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-)<sub>n</sub>,  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n represents an integer of from 1 to 3,  $R^9$  is a substituent group as defined above and  $R^{10}$  is defined as  $R^9$ .

In particular embodiments, the enhancing agent is 6-hydroxy-2-naphtoic acid, 7-methoxy-2-naphtol, 7-amino-2-20 naphthalene sulfonic acid, 5-amino-2-naphthalene sulfonic acid, 1,5-diaminonaphthalene, 7-hydroxy-1,2-naphthimidazole, 5-amino-2-naphthalenesulfonic acid, or 7-methoxy-2-naphtol.

The enhancing agent of the invention may be in free form or in the form of an addition salt.

#### 25 Methods of Oxidizing a Substrate

In another aspect, the invention provides a method of oxidizing a substrate with a laccase enzyme and in the presence of an enhancing agent.

The enhancing agent may be present in free form or 30 in the form of an addition salt.

The enhancing agent may be present in concentrations of from 0.01 to 500  $\mu M,$  more preferred 0.1 to 250  $\mu M,$  most preferred 0.5 to 100  $\mu M.$ 

Molecular oxygen from the atmosphere will usually be present in sufficient quantity.

### Laccase and Related Enzymes

The enhancing agents of the invention are agents capable of enhancing the activity of laccases and laccase related enzymes. In the context of this invention, laccases and laccase related enzymes contemplate any laccase enzyme comprised by the enzyme classification EC 1.10.3.2, any chatechol oxidase enzyme comprised by the enzyme classification EC 1.10.3.1, any bilirubin oxidase enzyme comprised by the enzyme classification EC 1.3.3.5 or any monophenol monooxygenase enzyme comprised by the enzyme classification EC 1.14.99.1.

Preferably, the laccase employed in the method of the invention is derived from a fungi such as Trametes, e.g. T. villosa or T. versicolor, Collybia, Fomes, Lentinus, Pleurotus, Rhizoctonia, e.g. R. solani, Aspergillus, Neurospora, Podospota, Phlebia, e.g. P. radiata (WO 92/01046), Coriolus, e.g. C. hirsitus (JP 2-238885), Myceliophthora, e.g. M. thermophila or Botrytis. Bilirubin oxidase may preferably be derived from Myrothecium, e.g. M. verrucaria.

The laccase or the laccase related enzyme may furthermore be one which is producible by a method comprising cultivating a host cell transformed with a recombinant DNA vector which carries a DNA sequence encoding said laccase as well as DNA sequences encoding functions permitting the expression of the DNA sequence encoding the laccase, in a culture medium under conditions permitting the expression of the laccase enzyme, and recovering the laccase from the culture.

### Determination of Laccase Activity (LACU)

Laccase activity is determined from the oxidation of syringaldazin under aerobic conditions. The violet colour produced is photometered at 530 nm. The analytical conditions are 19  $\mu$ M syringaldazin, 23.2 mM acetate buffer, pH 5.5, 30°C, 1 min. reaction time.

1 laccase unit (LACU) is the amount of enzyme that catalyses the conversion of 1.0  $\mu mole$  syringaldazin per minute at these conditions.

# Determination of Bilirubin Oxidase Activity (Sigma Units)

1 bilirubin oxidase Sigma unit is the amount of enzyme that will oxidize 1.0  $\mu$ mole of bilirubin per minute at pH 8.4 and 30°C.

#### Industrial Applications

In a preferred embodiment, the method of the invention finds application for bleaching of dye in solutions.

In another embodiment, the method of the invention finds application for dye transfer inhibition, e.g. for treatment of dyed textiles (cf. e.g. WO 92/18687) or during laundering (cf. e.g. WO 91/05839).

Accordingly, in a specific embodiment, the invention provides a method for inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the method comprising treatment of the wash liquor with a laccase or a laccase related enzyme in the presence of an enhancing agent. The textile dye may be a synthetic dye such as an azo dye, or a natural or nature-identical dye.

In a third embodiment, the method of the invention finds application in bleaching of pulp for paper production.

Accordingly, the invention provides a method for bleaching of lignin-containing material, in particular bleaching of pulp for paper production, which method comprises treatment of the lignin or lignin containing material with a laccase or a laccase related enzyme in the presence of an only on the invention.

In a fourth embodiment, the method of the invention finds application for lignin modification, e.g. in the manufacture of wood composites, e.g. wood fibre materials such as chipboards, fibre boards, or particle boards, or in the

manufacture of laminated wood products, such as laminated beams and plywood.

Accordingly, the invention provides a method for enzymatic polymerization and/or modification of lignin or slignin containing material, which method comprises treatment of the lignin or lignin containing material with a laccase or a laccase related enzyme in the presence of an enhancing agent of the invention.

In a fifth embodiment, the method of the invention 10 finds application in treatment of waste water e.g. waste water from the chemical or pharmaceutical industry, from dye manufacturing, from dye-works, from the textile industry, or from pulp production (cf. e.g. US 4,623,465, or JP-A-2-31887).

In a more specific aspect, the invention provides a method for treatment of waste water from dye manufacturing, from dye-works, from textile industry, or from pulp manufacturing, the method comprising treatment of the waste water with a laccase or a laccase related enzyme in the presence of an enhancing agent of the invention.

#### 20 <u>Detergent Compositions</u>

According to the invention, the enhancing agent may be added as a component of a detergent composition. As such, it may be included in the detergent composition in the form of a detergent additive. The detergent composition as well as the detergent additive may additionally comprise one or more other enzymes conventionally used in detergents, such as proteases, lipases, amylases, cutinases, cellulases and peroxidases.

In a specific aspect, the invention provides a detergent additive. The enzymes may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of these enzymes. A detergent additive of the invention, i.e. a separated additive or a combined additive, can be formulated e.g. as granulates, liquids, slurries, etc. Preferred detergent additive formulations are granulates, in particular non-dusting

granulates, liquids, in particular stabilized liquids, slurries, or protected enzymes.

Non-dusting granulates may be produced, disclosed in US 4,106,991 and 4,661,452 (both to Novo Industri 5 A/S) and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molar weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the 10 alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in patent GB 1483591. Liquid 15 enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Other enzyme stabilizers are well known in the art. Protected enzymes may be prepared according to the method disclosed in EP 20 238,216.

The detergent composition of the invention may be in any convenient form, e.g. as powder, granules, paste or liquid. A liquid detergent may be aqueous, typically containing up to 70 % water and 0-30 % organic solvent, or nonaqueous.

The detergent composition comprises one or more surfactants, each of which may be anionic, nonionic, cationic, or zwitterionic. The detergent will usually contain 0-50 % of anionic surfactant such as linear alkylbenzenesulfonate (LAS), alpha-olefinsulfonate (AOS), alkyl sulfate (fatty alcohol sulfate) (AS), alcohol ethoxysulfate (AEOS or AES), secondary alkanesulfonates (SAS), alpha-sulfo fatty acid methyl esters, alkyl- or alkenylsuccinic acid or soap. It may also contain 0-40 % of nonionic surfactant such as alcohol ethoxylate (AEO or AE), carboxylated alcohol ethoxylates, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamineoxide, ethoxylated fatty acid monoethanolamide, or

HEDDEID WAS DERVISENT

polyhydroxy alkyl fatty acid amide (e.g. as described in WO 92/06154).

The detergent composition may additionally comprise one or more other enzymes, such as amylases, lipases, cutinases, proteases, cellulases and peroxidases.

The detergent may contain 1-65 % of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTMPA), alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g. SKS-6 from Hoechst). The detergent may also be unbuilt, i.e. essentially free of detergent builder.

The detergent may comprise one or more polymers.

15 Examples are carboxymethylcellulose (CMC), poly(vinyl-pyrrolidone) (PVP), polyethyleneglycol (PEG), poly(vinyl-alcohol) (PVA), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

The detergent may contain a bleaching system which may comprise a  $H_2O_2$  source such as perborate or percarbonate which may be combined with a peracid-forming bleach activator such as tetraacetylethylenediamine (TAED) or nonanoyloxybenzenesulfonate (NOBS). Alternatively, the bleaching system may comprise peroxyacids of e.g. the amide, imide, or sulfone type.

The enzymes of the detergent composition of the invention may be stabilized using conventional stabilizing agents, e.g. a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative as e.g. an aromatic borate ester, and the composition may be formulated as described in e.g. WO 92/19709 and WO 92/19708.

The detergent may also contain other conventional detergent ingredients such as e.g. fabric conditioners instructured clays, foam boosters, suds suppressors, anti-corrosion

agents, soil-suspending agents, anti-soil redeposition agents, dyes, bactericides, optical brighteners, or perfume.

The pH (measured in aqueous solution at use concentration) will usually be neutral or alkaline, e.g. 7-11.

- Particular forms of detergent compositions within the scope of the invention include:
  - 1) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

10		linear alkylbenzenesulfonate (calculated as acid)	7	-	12%
	-	alcohol ethoxysulfate (e.g. C <sub>12-18</sub> alcohol, 1-2 EO) or alkyl sulfate (e.g. C <sub>16-18</sub> )	1	-	4%
15		alcohol ethoxylate (e.g. C <sub>14-15</sub> alcohol, 7 EO)	5	-	9%
	_	sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	14	-	20%
	-	soluble silicate (as Na <sub>2</sub> O, 2SiO <sub>2</sub> )	2	-	6%
	-	zeolite (as NaAlSiO <sub>4</sub> )	15	-	22%
	-	sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	0	-	6%
20		sodium citrate/citric acid (as C <sub>6</sub> H <sub>5</sub> Na <sub>3</sub> O <sub>7</sub> /C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> )			15%
	-	sodium perborate (as NaBO <sub>3</sub> .H <sub>2</sub> O)			18%
	-	TAED	2	-	6%
	~	carboxymethylcellulose	0	-	2%
25	-	polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	0	_	3%
	-	enzymes	0	-	5%
30		minor ingredients (e.g. suds suppressors, perfume, optical brightener, photobleach)	0	_	5%

- 2) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising
- linear alkylbenzenesulfonate
   (calculated as acid)

6 - 11%

	-	alcohol ethoxysulfate (e.g. C <sub>12-18</sub> alcohol, 1-2 EO) or alkyl sulfate (e.g. C <sub>16-18</sub> )	1	-	3%
5	-	alcohol ethoxylate (e.g. C <sub>14-15</sub> alcohol, 7 EO)	5	-	9%
	-	sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	15	-	21%
	-	soluble silicate (as Na <sub>2</sub> O, 2SiO <sub>2</sub> )	1	-	4%
	_	zeolite (as NaAlSiO <sub>4</sub> )	24	-	34%
	-	sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	4	-	10%
10	-	sodium citrate/citric acid	0	-	15 %
	-	(as C <sub>6</sub> H <sub>5</sub> Na <sub>3</sub> O <sub>7</sub> /C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> ) carboxymethylcellulose	0	-	2%
	-	polymers (e.g. maleic/acrylic acid copolymer PVP, PEG)	er, 1	-	6%
15	-	enzymes	0	-	5%
	-	minor ingredients (e.g. suds suppressors, perfume)	0	-	5%
		A detergent composition formulated as a galk density of at least 600 g/l comprising	ranu	la	te having a
20		linear alkylbenzenesulfonate (calculated as acid)	5	-	9%
	-	alcohol ethoxylate (e.g. C <sub>12-15</sub> alcohol, 7 EO)	7	-	14%
25		soap as fatty acid (e.g. C <sub>16-22</sub> )	1	-	3%
	-	sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	10	-	17%
	-	soluble silicate (as Na <sub>2</sub> O, 2SiO <sub>2</sub> )	3	-	9%
	-	zeolite (as NaAlSiO4)	23	-	33%
	-	sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	0	-	4%
30	_	sodium perborate (as NaBO3.H2O)	8	-	16%
	-	TAED	2	-	8%
	_	phosphonate (e.g. EDTMPA)	0	-	1%

	-	carboxymethylcellulose	O	-	28	
	-	polymers (e.g. maleic/acrylic acid copolym PVP, PEG)		-	3%	•
	-	enzymes	0	-	5%	
5	-	minor ingredients (e.g. suds suppressors, perfume, optical brightener)	. 0	-	5%	
	4 ) bi	A detergent composition formulated as a gall and the state of the stat	granu	la	ite ha	ving a
10		linear alkylbenzenesulfonate (calculated as acid)	8	_	12%	
	-	alcohol ethoxylate (e.g. C <sub>12-15</sub> alcohol, 7 EO)	10	-	25%	
	-	sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	14	-	22%	
	-	soluble silicate (as Na <sub>2</sub> O, 2SiO <sub>2</sub> )	1	-	5%	
15	-	zeolite (as NaAlSiO <sub>4</sub> )	25	-	35%	
	-	sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	0	-	10%	
	<u>.</u>	carboxymethylcellulose	. 0	· <b>-</b>	2%	
	-	polymers (e.g. maleic/acrylic acid copolymer PVP, PEG)		-	3%	
20	-	enzymes	0	-	5%	
	-	minor ingredients (e.g. suds suppressors, perfume)	0	-	5%	
		An aqueous liquid detergent composition collinear alkylbenzenesulfonate	ompri	is	ing	
25		(calculated as acid)	15	-	21%	
	-	alcohol ethoxylate (e.g. C <sub>12-15</sub> alcohol, 7 EO or C <sub>12-15</sub> alcohol, 5 EO)	12	-	18%	
	-	soap as fatty acid (e.g. oleic acid)	3	-	13%	
30	-	alkenylsuccinic acid (C <sub>12-14</sub> )	0	-	13%	
	_	aminoethanol	8	-	18%	

	-	citric acid	2	-	8%	
	-	phosphonate	0	-	3%	
	_	polymers (e.g. PVP, PEG)	0	-	3%	
	_	borate (as B <sub>4</sub> O <sub>7</sub> )	0	-	2%	
5	_	ethanol	0	-	3%	
	_	propylene glycol	8	-	14%	
	-	enzymes	0	-	5%	
10		<pre>minor ingredients (e.g. dispersants, suds suppressors,   perfume, optical brightener)</pre>	0	-	5%	
	6) ir	An aqueous structured liquid detergent compo	si	ti	on co	mpris-
		linear alkylbenzenesulfonate	15	-	21%	
15	-	alcohol ethoxylate (e.g. C <sub>12-15</sub> alcohol, 7 EO or C <sub>12-15</sub> alcohol, 5 EO)	3	-	9%	
	_	soap as fatty acid (e.g. oleic acid)	3	-	10%	
	_	zeolite (as NaAlSiO4)	14	-	22%	
20	-	potassium citrate	9	-	18%	
	_	borate (as B <sub>4</sub> O <sub>7</sub> )	0	-	2%	
	-	carboxymethylcellulose	0	-	2%	
	-	polymers (e.g PEG, PVP)	0	-	3%	
25	-	anchoring polymers as e.g. lauryl methacrylate/acrylic acid copoly molar ratio 25:1; MW 3800	me: 0	r; -	3%	
	_	glycerol	0	-	5%	
	-	enzymes	0	-	5%	
30	-	<pre>minor ingredients (e.g. dispersants, suds suppressors, perfume   optical brighteners)</pre>	, 0	_	5%	

7) A detergent composition formulated as a gr	anulate having a
bulk density of at least 600 g/l comprising	
- fatty alcohol sulfate	5 - 10%
- ethoxylated fatty acid monoethanolamide	3 - 9%
5 - soap as fatty acid	0 - 3%
- sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	5 - 10%
- soluble silicate (as Na <sub>2</sub> O,2SiO <sub>2</sub> )	1 - 4%
- zeolite (as NaAlSiO <sub>4</sub> )	20 - 40%
- sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	2 - 8%
10 - sodium perborate (as NaBO <sub>3</sub> .H <sub>2</sub> O)	12 - 18%
- TAED	2 - 7%
<ul> <li>polymers (e.g. maleic/acrylic acid copolyme PEG)</li> </ul>	r, 1 - 5%
- enzymes	0 - 5%
<pre>15 - minor ingredients (e.g. optical brightener, suds suppressors, perfume)</pre>	0 - 5%
8) A detergent composition formulated as a gran	nulate comprising
<ul> <li>linear alkylbenzenesulfonate (calculated as acid)</li> </ul>	8 - 14%
20 - ethoxylated fatty acid monoethanolamide	5 - 11%
- soap as fatty acid	0 - 3%
- sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	4 - 10%
- soluble silicate (as Na <sub>2</sub> O,2SiO <sub>2</sub> )	1 - 4%
- zeolite (as NaAlSiO4)	30 - 50%
25 - sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	3 - 11%
- sodium citrate (as C <sub>6</sub> H <sub>5</sub> Na <sub>3</sub> O <sub>7</sub> )	5 - 12%
<ul> <li>polymers (e.g. PVP, maleic/acrylic acid copolymer, PEG)</li> </ul>	1 - 5%
- enzymes	0 - 5%
30 - minor ingredients (e.g. suds suppressors,	

29

		perfume)	0	-	5%
	9	) A detergent composition formulated as a gra	nula	ite	comprising
	-	linear alkylbenzenesulfonate (calculated as acid)	6	-	12%
5	-	nonionic surfactant,	1	-	4%
	-	soap as fatty acid	. 2	-	6%
	-	sodium carbonate (as Na <sub>2</sub> CO <sub>3</sub> )	14	_	22%
	-	zeolite (as NaAlSiO4)	18	-	32%
	-	sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	. 5	-	20%
10	-	sodium citrate (as C <sub>6</sub> H <sub>5</sub> Na <sub>3</sub> O <sub>7</sub> )	3	-	8%
	-	sodium perborate (as NaBO3.H2O)	4	-	9%
	-	bleach activator (e.g. NOBS or TAED)	1	-	5%
	-	carboxymethylcellulose	0	-	2%
	-	polymers (e.g. polycarboxylate or PEG)	1	-	5%
15	-	enzymes	0	-	5%
	-	<pre>minor ingredients (e.g. optical brightener, perfume)</pre>	0	-	5%
	10	)) An aqueous liquid detergent composition (	compi	ris	sing
20		linear alkylbenzenesulfonate (calculated as acid)	15	-	23%
	-	alcohol ethoxysulfate (e.g. C <sub>12-15</sub> alcohol, 2-3 EO)	8	-	15%
25	-	alcohol ethoxylate (e.g. C <sub>12-15</sub> alcohol, 7 EO or C <sub>12-15</sub> alcohol, 5 EO)	3	-	9%
	-	soap as fatty acid (e.g. lauric acid)	0	-	3%
	-	aminoethanol 1 - 5%			
	-	sodium citrate	5	-	10%
	-	hydrotrope (e.g. sodium toluenesulfonate)	2	-	6%

	-	borate (as B <sub>4</sub> O <sub>7</sub> )	0	-	2%
	-	carboxymethylcellulose	0	-	1%
	-	ethanol	1	-	3%
	-	propylene glycol	2	-	5%
5	-	enzymes	0	-	5%
	-	minor ingredients (e.g. polymers, dispers perfume, optical brighteners)	ants, 0	-	5%
	11	An aqueous liquid detergent composition	comp	ri	sing
10		linear alkylbenzenesulfonate (calculated as acid)	20	-	32%
	-	alcohol ethoxylate (e.g. C <sub>12-15</sub> alcohol, 7 EO or C <sub>12-15</sub> alcohol, 5 EO)	6	_	12%
	-	aminoethanol 2 - 6	ે		
15	-	citric acid	8	-	14%
	-	borate (as B <sub>4</sub> O <sub>7</sub> )	1	-	3%
		polymer (e.g. maleic/acrylic acid copolym anchoring polymers as e.g. lauryl methacrylate/acrylic acid		_	3%
20		copolymer and CMC)			8%
		glycerol	_		5%
		enzymes	U	-	56
	-	minor ingredients (e.g. hydrotropes, dispersants, perfume, optical brighteners	) 0	-	5%
25	12	2) A detergent composition formulated as a bulk density of at least 600 g/l compri		.la	ate having a
30		anionic surfactant (linear alkylbenzenesulfonate, alkyl sulfate, alp olefinsulfonate, alpha-sulfo fatty acid methyl esters, alkanesulfonates, soap)	ha-	-	40%
	-	nonionic surfactant (e.g. alcohol ethoxylate)	1	-	10%
	_	sodium carbonate (as Na <sub>2</sub> CO <sub>2</sub> )	8	-	25%

	-	soluble silicates (as Na <sub>2</sub> O, 2SiO <sub>2</sub> )	5	-	15%
	-	sodium sulfate (as Na <sub>2</sub> SO <sub>4</sub> )	0	-	5%
	-	zeolite (as NaAlSiO4)	15	-	28%
	-	sodium perborate (as NaBO <sub>3</sub> .4H <sub>2</sub> O)	Ö	-	20%
5	-	bleach activator (TAED or NOBS)	0	-	5%
	-	enzymes	0	-	5%
	-	minor ingredients (e.g. perfume, optical brighteners)	0	-	3%

- 13) Detergent formulations as described in 1) 12) where the 10 content of linear alkylbenzenesulfonate or a part of it is substituted by alkyl sulfate  $(C_{12}-C_{18})$ .
- 14) Detergent formulations as described in 1) 13) which contain a stabilized or encapsulated peracid either as an additional component or as a substitute for already specified 15 bleach systems.
  - 15) Detergent compositions as described in 3), 7), 9) and 12) where the content of perborate is substituted with percarbonate.
- 16) Detergent composition formulated as a nonaqueous detergent 20 liquid comprising a liquid nonionic surfactant as e.g. linear alkoxylated primary alcohol, a builder system (e.g. phosphate), enzyme and alkali. The detergent may also comprise anionic surfactant and/or a bleach system.

The following examples further illustrate the present invention, and they are not intended to be in any way limiting to the scope of the invention as claimed.

#### EXAMPLE 1

32

# Bleaching of Direct Blue 1 with Bilirubin Oxidase and 10-Methylphenothiazine

The bleaching of Direct Blue 1 (DB1) by bilirubin oxidase obtained from Sigma with and without 10-methyl-pheno-5 thiazine (MPT) is presented in the attached Fig. 2.

The following conditions were used:

Final concentrations

Buffer 50 mM phosphate, pH 7

MPT 0, 2.5, 5, or 10  $\mu$ M

10 DB1  $\approx$  Abs<sub>605rm</sub> = 0.6

Bilirubin oxidase 0.027 Sigma units/ml

The experiment was started by the addition of enzyme, and the absorbance at  $605~\mathrm{nm}$  was monitored for  $5~\mathrm{minutes}$ . The temperature was  $25~\mathrm{C}$ .

No attempt to maintain a constant concentration of dissolved oxygen was made.

As evidenced by Fig. 2, bleaching is enhanced by increasing concentrations of the enhancer of the invention.

#### EXAMPLE 2

#### 20 Bleaching of Direct Blue 1

Bilirubin oxidase: Myrothecium verrucaria bilirubin oxidase was purchased from Sigma (B-0390). A stock solution was made containing 27 Sigma units/ml, which was stored in the freezer until use.

Laccase obtained from Trametes villosa: 800 ml culture broth of Trametes villosa, CBS 678.70, was filtered with filter aid to give a clear filtrate, which was concentrated and washed by ultrafiltration on a membrane with a cut-off of 6-8 kDa. One ml samples of concentrated preparation was applied onto a Q-Sepharose HP column (Pharmacia, Sweden) equilibrated with 0.1 M fosfate pH 7, and the laccase was eluted with a flat NaCl

gradient around 0.25 M. Fractions with laccase activity from 10 runs were pooled and concentrated by ultrafiltration to an activity of 500 LACU/ml.

Enhancers: Chemicals were obtained from Sigma-Aldrich, Janssen 5 Chimica, Kodak, Tokyo Kasai Organic Chemicals or Daiichi Pure Chemicals Co. N-methylated derivatives of phenothiazine and phenoxazine were made by methylation with methyliodide as described by Cornel Bodea and Ioan Silberg in "Recent Advances in the Chemistry of Phenothiazines" (Advances in heterocyclic chemistry, 1968, Vol. 9, pp. 321-460); B. Cardillo & G. Casnati in Tetrahedron, 1967, Vol. 23, p. 3771. Phenothiazine and phenoxazine propionic acids may be prepared as described in J. Org. Chem. 15, 1950, pp. 1125-1130. Hydroxyethyl and hydroxypropyl derivatives of phenothiazine and phenoxazine may be prepared as described by G. Cauquil in Bulletin de la Society Chemique de France, 1960, p.1049.

Due to low solubility some of the enhancers were dissolved in a small volume of ethanol before dilution in water.

The following conditions were used:

20 Final concentration

400 µl 50 mM Britton-Robinson buffer,

pH 5.5 and pH 7.0 respectively, 20 mM 200  $\mu$ l DB1 ~ 3.0 Abs. Units (610 nm) 0.6 (A<sub>610rm</sub>) 200  $\mu$ l 50  $\mu$ M enhancer 10  $\mu$ M

25 200  $\mu$ l Enzyme

Reagents were mixed in a 1 cm thermostated cuvette at  $30\,^{\circ}\text{C}$  and the bleaching was started by addition of enzyme.

The bleaching was detected spectrophotometrically at 610 nm, which is the absorption peak of DB1. After 5 sec. 30 bleaching was followed for 4 minutes.

From the results presented in Table 1-2, below, it appears that adding enhancers of the invention a much faster bleaching of the dye can be obtained compared to the experiment without enhancer. Enzyme dosages given are in the final incubation mixture.

Table 1
Bleaching of Direct Blue 1 with Trametes villosa laccase,
obtained as described above, at pH 5.5 (0.1 LACU/ml) and pH 7.0
(1 LACU/ml).\_

5		(-∆mAbs/	in 4 minut 4 min) H 7.0	es	
	No enhancer	0	0		
10	10-Phenothiazine- propionic acid	387	182		
	10-Methyl- phenothiazine	222	22		
15	4'-Hydroxy- 4-biphenyl- carboxylic acid	46	34		
20	4,4'- Diaminostilbene- 2,2'-disulfonic acid	3.5	6		
	6-Hydroxy-2- naphthoic acid	12	20		
	10-Methylphenoxa- zine	364	296		
25	10-Phenoxazinepro- pionic acid	364	268		
	10-Ethyl-4-pheno- thiazinepropionic acid	411	175		
30	10-(2-Hydroxyethyl)- phenothiazine	373	184		
	10-(2-Hydroxyethyl)- phenoxazine	429	192		
35	10-(3-Hydroxypropyl)- phenothiazine	428	219		

4,4'-Dimethoxy-N-

PCT/DK94/00210

35

methyl-diphenylamine	210	113
Vanillin azine	118	5

Table 2
Bleaching of Direct Blue 1 with Myrothecium verrucaria bilirubin oxidase, obtained as described above, at pH 5.5 (0.00675
Sigma units/ml) and pH 7.0 (0.0675 Sigma units/ml).

10	Enhancer		aching in 4 minutes AmAbs/4 min) pH 7.0	
10				
	No enhancer	68	207	
	10-Phenothiazine- propionic acid	95	244	
15	10-Methyl- phenothiazine	76	250	
	10-Methylphenoxa- zine	143	374	
	10-Phenoxazinepro- pionic acid	96	347	
20	10-Ethyl-4-pheno- thiazinepropionic acid	103	293	

COUCH SHO BENT SERT

36

#### CLAIMS

1. A method of oxidizing a substrate with an enzyme selected from the group consisting of laccase, chatechol oxidase, monophenol monooxygenase and bilirubin oxidase, characterized by the presence of an enhancing agent being an organic chemical compound consisting of at least two aromatic rings, of which aromatic rings at least one is substituted with one or more of the following atoms: nitrogen, oxygen, and sulfur; and which aromatic rings may furthermore be fused 10 rings.

2. A method according to claim 1 in which the enhancing agent is described by the general formula I:

in which general formula A represents a single bond, or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-CR^{11}=N-N=CR^{15}-)$ , 15  $(-NR^{11}-)$ ,  $(-CR^{12}=N-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-)_n$ ,  $(-CR^{12}=N-NR^{11}-)$ ,  $(-NR^{11}-CR^{12}=CR^{13}-)$ , (-N=N-), in which groups n represents an integer of from 1 to 6; or A represents carbon, carbonyl, nitrogen, sulfur, oxygen, selenium, or phosphor, which carbon, phosphor and nitrogen may be unsubstituted or substituted with a substituent group  $R^{11}$ ;

and in which general formula the substituent groups  $R^1-R^{13}$  and  $R^{15}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, sulfandiyl, nitro, amino, phenyl,  $C_1-C_1$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl, aryl- $C_1-C_5$ -alkyl; which carbamoyl, sulfamoyl, and

amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl, and aryl- $C_1-C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

which substituent group  $R^{14}$  represents any of the 10 following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; carbamoyl, sulfamoyl, and amino groups may furthermore be 15 unsubstituted or substituted once or twice with hydroxy,  $C_1 - C_5$ alkyl,  $C_1$ - $C_5$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; 20 and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, 25 and sulfamoyl;

or in which general formula R<sup>5</sup> and R<sup>6</sup> may together form a group -B-, in which B represents a single bond, the group (-CH=CH-)<sub>n</sub>, or the group (-CH=N-)<sub>n</sub>, in which groups n represents an integer of from 1 to 6; or B represents carbon, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group R<sup>14</sup> as defined above;

or in which general formula two of the substituent groups  $R^1-R^{10}$  may together form a group -C-, in which C respectively. The substituent groups any of the following groups:  $(-CHR^{11}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ , in which groups n represents an integer of from 2 to

4, and in which groups  $R^{11}$  is a substituent group as defined above;

or in which general formula  $R^5$  and  $R^{12}$ , and/or  $R^6$  and  $R^{13}$ , when n in the above formula being 1, may together form a group -D-, in which D represents the groups:  $(-CHR^{11}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{11}=CR^{15}-)$ ,  $(-CR^{11}=N-)$ ,  $(-N=CR^{11}-)$ , (-O-), (>C=O) or (-S-), and in which groups  $R^{11}$  and  $R^{15}$  are substituent groups as defined above.

3. A method according to claim 1, in which the 10 enhancing agent is described by the general formula II:

in which the substituent groups R<sup>1</sup>-R<sup>8</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, and amino groups may furthermore be unsubstituted or substituted or substituted or substituted or substituted with one or more substituent groups R<sup>9</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>;

which substituent group R<sup>9</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof,

sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent 15 groups  $R^1-R^8$  may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-), (-CH=CH-),  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n represents an integer of from 1 to 3,  $R^9$  is a substituent group as defined above and  $R^{10}$  is defined as  $R^9$ .

4. A method according to either of claims 1-2, in which the enhancing agent is described by the following formula:

in which formula E represents a single bond, a carbonyl group or one of the following groups:  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or  $(-NR^{11}-)$ , in which n represents an integer from 1 to 2. The substituents groups  $R^1-R^{11}$  may be identical or different, independently being one of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, car-

bamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>14</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>14</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsubstituted, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>14</sup>;

which substituent group R14 represents any of the following radicals: hydroxy, formyl, carboxy and esters and 15 salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof. nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C1-C3-20 alkyl,  $C_1 - C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be 25 saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which specific formula two of the substituent groups  $R^5$  and  $R^6$  may together form a group -B-, in which B represents the groups:  $(-CH=N-)_n$ , (-CH=CH-) or  $(-CHR^{14}-)$  in which groups n represents an integer of from 1 to 2 and  $R^{14}$  is a substituent group as defined above.

41

5. A method according to claim 4, in which the enhancing agent is 4-amino-4'-methoxystilbene, 4,4'-diamino-stilbene-2,2'-disulfonic acid, iminostilbene, 4,4'-dihydroxybenzophenone, N-benzylidene-4-biphenylamine, 4,4'-diaminodi-5 phenylamine, 4,4'-dimethoxy-N-methyl-diphenylamine, 2,7-diaminofluorene or triphenylamine.

6. A method according to either of claims 1-2, in which the enhancing agent is described by the following formula:

in which formula X represents one of the following groups: (-O-), (-S-), (-NR<sup>15</sup>-), (-CHR<sup>15</sup>-), (>C=O), (-CH=CH-), (-CH=N-), (-N=N-) and the substituent groups R<sup>1</sup>-R<sup>9</sup> and R<sup>15</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and setters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted or substituted or substituted with one or more substituent groups R<sup>10</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups

may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{10}$ ;

which substituent group  $R^{10}$  represents any of the 5 following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino, C,-C5-alkyl, C,-C5-alkoxy; which 10 carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_5$ alkyl,  $C_1$ - $C_5$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, 15 carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters 20 and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups  $R^1-R^8$  may together form a group -B-, in which B represents any of the following the groups: (-CHR<sup>10</sup>-N=N-), (-25 CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub> or (-N=CR<sup>10</sup>-NR<sup>11</sup>-), in which groups n represents an integer of from 1 to 3,  $R^{10}$  is a substituent group as defined above and  $R^{11}$  is defined as  $R^{10}$ .

- 7. A method according to claim 6, in which X in the formula is (-0-) or (-S-) and  $R^5$  in the formula is an alkyl 30 group which may be further substituted.
  - 8. A method according to either of claims 6-7, in which the enhancing agent is 10-methylphenothiazine, 10-phenothiazine-propionic acid, N-hydroxysuccinimide-10-phenothiazine-propionate, 10-ethyl-4-phenothiazine-carboxylic acid,

10-ethylphenothiazine, 10-propylphenothiazine, 10-isopropyl-phenothiazine, methyl-10-phenothiazinepropionate, 10-phenyl-phenothiazine, 10-allylphenothiazine, 10-(3-(4-methyl-1-piperazinyl)propyl)phenothiazine, 10-(2-pyrrolidinoethyl)-phenothiazine, 2-acetyl-10-methylphenothiazine, 4-carboxy-10-phenothiazine-propionic acid, 10-methylphenoxazine, 10-ethyl-phenoxazine, 10-phenoxazine-propionic acid, 4-carboxy-10-phenoxazine-propionic acid, 10-(2-hydroxyethyl)phenothiazine, 10-(2-hydroxyethyl)phenothiazine,

9. A method according to either of claims 1-2, in which the enhancing agent is a biphenyl derivative of the following formula:

in which the substituent groups R<sup>1</sup>-R<sup>10</sup>, which may be identical or different, independently represents any of the 15 following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>11</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>11</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl,

 $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{11}$ ;

- which substituent group  $R^{11}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; 10 which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>alkyl,  $C_1$ - $C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, 15 carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters 20 and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl.
- 10. A method according to claim 9, in which the enhancing agent is benzidine, 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine, 3,3',5,5'-tetramethylbenzidine, 4'-hydroxy-25 4-biphenylcarboxylic acid, or 4,4'-dihydroxybiphenylene.
- 11. A method according to either of claims 1-2, in which the enhancing agent is 2-(p-aminophenyl)-6-methyl-benzothiazole-7-sulfonic acid, N-(4-(dimethylamino)benzylide-ne)-p-anisidine, 3-methyl-2-benzothiazolinone(4-(dimethylami-30 no)benzylidene)hydrazone or vanillin azine.
  - 12. A method according to either of claims 1 and 3, in which the enhancing agent is a naphthalene derivative of the following formula:

in which the substituent groups R<sup>1</sup>-R<sup>8</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>9</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>;

which substituent group R<sup>9</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters

and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups  $R^1-R^8$  may together form a group -B-, in which B respected any of the following groups: (-N=N-), (-CH=CH-), (-CH=CH-), (-CH=N-),  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n represents an integer of from 1 to 3,  $R^9$  is a substituent group as defined above and  $R^{10}$  is defined as  $R^9$ .

- 13. A method according to claim 12, in which the enhancing agent is 6-hydroxy-2-naphtoic acid, 7-methoxy-2-naphtol, 7-amino-2-naphthalene sulfonic acid, 5-amino-2-naphthalene sulfonic acid, 1,5-diaminonaphthalene, 7-hydroxy-1,2-naphthimidazole, 5-amino-2-naphthalenesulfonic acid, or 7-methoxy-2-naphtol.
- 14. A method according to any of claims 1-13, in which said method is a method for bleaching of dye in solutions.
- 15. A method according to any of claims 1-13, in which said method is a method for inhibiting the transfer of a 20 textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor.
- 16. A method according to any of claims 1-13, in which said method is a method for bleaching of lignin-containing material, in particular bleaching of pulp for paper production.
  - 17. A method according to any of claims 1-13, in which said method is a method for enzymatic polymerization and/or modification of lignin or lignin containing material.
- 18. Use of the method according to claim 17 in the 30 manufacture of wood composites such as chipboards, fibre

boards, and particle boards, or in the manufacture of laminated wood products such as laminated beams and plywood.

- 19. A method according to any of claims 1-13, in which said method is a method for treatment of waste water, in particular waste water from the pharmaceutical or chemical industry, e.g. waste water from dye manufacturing, from textile industry, or from pulp manufacturing.
- 20. A method according to any of claims 14-19, in which the enhancing agent is added at the beginning of, or 10 during the process.
  - 21. A method according to any of claims 14-20, in which the amount of enhancing agent is in the range of from 0.01-500  $\mu\text{M}$ , more preferred 0.1-250  $\mu\text{M}$ , most preferred 0.5-100  $\mu\text{M}$ .
- 15 22. A method according to any of claims 14-21, in which the laccase enzyme is derived from <u>Trametes</u>, e.g. <u>T. versicolor or T. villosa</u>, <u>Collybia</u>, <u>Fomes</u>, <u>Lentinus</u>, <u>Pleurotus</u>, <u>Rhizoctonia</u>, e.g. <u>R. solani</u>, <u>Aspergillus</u>, <u>Neurospora</u>, <u>Podospora</u>, <u>Phlebia</u>, <u>Myceliopthora</u>, e.g. <u>M. thermophila</u>, <u>Botrytis</u>, or the bilirubin oxidase is derived from <u>Myrothecium</u>, e.g. <u>M. verrucaria</u>.
- 23. A detergent additive capable of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the detergent additive comprising an enzyme exhibiting laccase activity, characterized by the presence of an enhancing agent being an organic chemical compound consisting of at least two aromatic rings, of which aromatic rings at least one is substituted with one or more of the following atoms: nitrogen, oxygen, and sulfur; and which aromatic rings may furthermore be fused rings.

48

24. A detergent additive according to claim 23, in which the enhancing agent is described by the general formula I:

in which general formula A represents a single bond, 5 or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-CR^{11}=N-N=CR^{15}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{12}=N-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-)_n$ ,  $(-CR^{12}=N-NR^{11}-)$ ,  $(-NR^{11}-CR^{12}=CR^{13}-)$ , (-N=N-), in which groups n represents an integer of from 1 to 6; or A represents carbon, carbonyl, nitrogen, sulfur, oxygen, selenium, or tellurium, 10 which carbon and nitrogen may be unsubstituted or substituted with a substituent group  $R^{11}$ ;

and in which general formula the substituent groups  $R^{1}-R^{13}$  and  $R^{15}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, 15 hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl,  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl,  $aryl-C_1-C_5-alkyl;$  which carbamoyl, sulfamoyl, and amino groups 20 may furthermore be unsubstituted or substituted once or twice with a substituent group R14; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ alkyl, and  $aryl-C_1-C_5$ -alkyl groups may be saturated or un-25 saturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R14;

which substituent group  $R^{14}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and

salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; which 5 carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_5$ alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, 10 carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C,-C,-alkyl, and C,-C,-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters 15 and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula R<sup>5</sup> and R<sup>6</sup> may together form a group -B-, in which B represents a single bond, the group (-CH=CH-)<sub>n</sub>, or the group (-CH=N-)<sub>n</sub>, in which groups n represents an integer of from 1 to 6; or B represents carbon, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group R<sup>14</sup> as defined above;

or in which general formula two of the substituent 25 groups  $R^1-R^{10}$  may together form a group -C-, in which C represents any of the following groups:  $(-CHR^{11}-N=N-)$ , (-CH=CH-), (-CH=N-), in which groups n represents an integer of from 2 to 4, and in which groups  $R^{11}$  is a substituent group as defined above;

or in which general formula  $R^5$  and  $R^{12}$ , and/or  $R^6$  and  $R^{13}$ , when n in the above formula being 1, may together form a group -D-, in which D represents the groups: (-CHR<sup>11</sup>-), (-NR<sup>11</sup>-), (-CR<sup>11</sup>=CR<sup>15</sup>-), (-CR<sup>11</sup>=N-), (-N=CR<sup>11</sup>-), (-O-), (>C=O) or (-S-), and in which groups  $R^{11}$  and  $R^{15}$  are substituent groups as defined 35 above.

25. A detergent additive according to claim 23, in which the enhancing agent is described by the general formula II:

in which the substituent groups R¹-R³, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C¹-C¹-alkyl, C¹-C⁵-10 alkoxy, carbonyl-C¹-C⁵-alkyl, aryl-C¹-C⁵-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R³; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R³; and which C¹-C¹-alkyl, C¹-C⁵-15 alkoxy, carbonyl-C¹-C⁵-alkyl, and aryl-C¹-C⁵-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R³;

which substituent group R° represents any of the 20 following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-

alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof,

or in which general formula two of the substituent groups  $R^1-R^8$  may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-), (-CH=CH-), (-CH=N-),  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n represents an integer of from 1 to 3,  $R^9$  is a substituent group as defined above and  $R^{10}$  is defined as  $R^9$ .

26. A detergent additive according to either of claims 23-24, in which the enhancing agent is described by the following formula:

in which formula E represents a single bond, a carbonyl group or one of the following groups:  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or  $(-NR^{11}-)$ , in which n represents an integer from 1 to 2. The substituents groups  $R^1-R^{11}$  may be identical or different, independently being one of the following radicals: hydrogen, bydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl,  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl,

aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

which substituent group R14 represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; 15 which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>alkyl, C,-C,-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, 20 carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters 25 and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which specific formula two of the substituent groups  $R^5$  and  $R^6$  may together form a group -B-, in which B represents the groups:  $(-CH=N-)_n$ , (-CH=CH-) or  $(-CHR^{14}-)$  in which 30 groups n represents an integer of from 1 to 2 and  $R^{14}$  is a substituent group as defined above.

27. A detergent additive according to claim 26, in which the enhancing agent is 4-amino-4'-methoxystilbene, 4,4'-diaminostilbene-2,2'-disulfonic acid, iminostilbene, 4,4'-35 dihydroxybenzophenone, N-benzylidene-4-biphenylamine, 4,4'-

diaminodiphenylamine, 4,4'-dimethoxy-N-methyl-diphenylamine, 2,7-diaminofluorene or triphenylamine.

28. A detergent additive according to either of claims 23-24, in which the enhancing agent is described by the following formula:

in which formula X represents one of the following groups: (-O-), (-S-),  $(-NR^{15}-)$ ,  $(-CHR^{15}-)$ , (>C=O), (-CH=CH-), (-CH=CH-)CH=N-) and the substituent groups  $R^1-R^9$  and  $R^{15}$ , which may be identical or different, independently represents any of the 10 following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl,  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, 15 sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{10}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>10</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C,-C,-alkoxy, carbonyl-C,-C,-alkyl, and aryl-C,-C,-alkyl groups 20 may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R10;

which substituent group  $R^{10}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and s salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino, C,-C,-alkyl, C,-C,-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_5$ alkyl, C,-C,-alkoxy; and which phenyl may furthermore be 10 substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamovl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may 15 furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent 20 groups  $R^1-R^8$  may together form a group -B-, in which B represents any of the following the groups:  $(-CHR^{10}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or  $(-N=CR^{10}-NR^{11}-)$ , in which groups n represents an integer of from 1 to 3,  $R^{10}$  is a substituent group as defined above and  $R^{11}$  is defined as  $R^{10}$ .

- 29. A detergent additive according to claim 28, in which X in the formula is (-O-) or (-S-) and R<sup>5</sup> in the formula is an alkyl group which may be further substituted.
- 30. A detergent additive according to either of claims 28-29, in which the enhancing agent is 10-methylpheno30 thiazine, 10-phenothiazine-propionic acid, N-hydroxysuccinimide-10-phenothiazine-propionate, 10-ethyl-4-phenothiazinecarboxylic acid, 10-ethylphenothiazine, 10-propylphenothiazine,
  10-isopropylphenothiazine, methyl-10-phenothiazinepropionate,
  10-phenylphenothiazine, 10-allylphenothiazine, 10-(3-(4-methyl-

1-piperazinyl)propyl)phenothiazine, 10-(2-pyrrolidinoethyl)-phenothiazine, 2-acetyl-10-methylphenothiazine, 10-methyl-phenoxazine, 10-(2-hydroxyethyl)phenothiazine, 10-(2-hydroxyethyl)phenoxazine or 10-(3-hydroxypropyl)phenothiazine.

31. A detergent additive according to either of claims 23-24, in which the enhancing agent is a biphenyl derivative of the following formula:

in which the substituent groups R<sup>1</sup>-R<sup>10</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>1</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituted group R<sup>11</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>11</sup>; and which C<sub>1</sub>-C<sub>1</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>11</sup>;

which substituent group R<sup>11</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and 25 salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be

unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_3$ -alkyl,  $C_1$ - $C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl.

- 32. A detergent additive according to claim 31, in which the enhancing agent is benzidine, 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine, 3,3',5,5'-tetramethylbenzidine, 4'-15 hydroxy-4-biphenylcarboxylic acid, or 4,4'-dihydroxybiphenylene.
- 33. A detergent additive according to either of claims 23-24, in which the enhancing agent is 2-(p-amino-phenyl)-6-methylbenzothiazole-7-sulfonic acid, N-(4-(dimethyl-20 amino)benzylidene)-p-anisidine, 3-methyl-2-benzothiazolinone(4-(dimethylamino)benzylidene)hydrazone or vanillin azine.
  - 34. A detergent additive according to either of claims 23 and 25, in which the enhancing agent is a naphthalene derivative of the following formula:

in which the substituent groups  $R^1-R^8$ , which may be identical or different, independently represents any of the

following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-salkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>9</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>;

which substituent group R9 represents any of the 15 following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be 20 unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>alkyl, C1-C3-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; 25 and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, 30 and sulfamoyl;

or in which general formula two of the substituent groups  $R^1-R^8$  may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-), (-CH=N-),  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n represents an integer of from 1 to 3,  $R^9$  is a substituent group as defined above and  $R^{10}$  is defined as  $R^9$ .

58

35. A detergent additive according to claim 34, in which the enhancing agent is 6-hydroxy-2-naphtoic acid, 7-methoxy-2-naphtol, 7-amino-2-naphthalene sulfonic acid, 5-amino-2-naphthalene sulfonic acid, 1,5-diaminonaphthalene, 7-5 hydroxy-1,2-naphthimidazole, 5-amino-2-naphthalenesulfonic acid, or 7-methoxy-2-naphtol.

36. A detergent additive according to any of claims 23-35, provided in the form of a granulate, preferably a non-dusting granulate, a liquid, in particular a stabilized liquid, 10 a slurry, or a protected enzyme.

37. A detergent composition capable of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the detergent composition comprising an enzyme exhibiting laccase activity, characterized by the presence of an enhancing agent being an organic chemical compound consisting of at least two aromatic rings, of which aromatic rings at least one is substituted with one or more of the following atoms: nitrogen, oxygen, and sulfur; and which aromatic rings may furthermore be fused rings.

38. A detergent composition according to claim 37, in which the enhancing agent is described by the general formula I:

in which general formula A represents a single bond, 25 or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-CR^{11}=N-N=CR^{15}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{12}=N-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-)_n$ ,  $(-R^{11}-CR^{12}=N-)_n$ ,  $(-R^{11}-CR^{12}=N-)_n$ , in which groups n

represents an integer of from 1 to 6; or A represents carbon, carbonyl, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group R<sup>11</sup>;

and in which general formula the substituent groups  $R^{1}-R^{13}$  and  $R^{15}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, 10 phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl,  $aryl-C_1-C_5-alkyl;$  which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R14; and which phenyl may furthermore 15 be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ alkyl, and  $aryl-C_1-C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent 20 groups R<sup>14</sup>;

which substituent group R14 represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and 25 salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C1-C5alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; and which phenyl may furthermore be 30 substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may 35 furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters

and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula R<sup>5</sup> and R<sup>6</sup> may together form a group -B-, in which B represents a single bond, the group (-CH=CH-)<sub>n</sub>, or the group (-CH=N-)<sub>n</sub>, in which groups n represents an integer of from 1 to 6; or B represents carbon, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group R<sup>14</sup> as defined above;

or in which general formula two of the substituent groups  $R^1-R^{10}$  may together form a group -C-, in which C represents any of the following groups:  $(-CHR^{11}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ , in which groups n represents an integer of from 2 to 4, and in which groups  $R^{11}$  is a substituent group as defined 15 above;

or in which general formula  $R^5$  and  $R^{12}$ , and/or  $R^6$  and  $R^{13}$ , when n in the above formula being 1, may together form a group -D-, in which D represents the groups: (-CHR<sup>11</sup>-), (-NR<sup>11</sup>-), (-CR<sup>11</sup>=CR<sup>15</sup>-), (-CR<sup>11</sup>=N-), (-N=CR<sup>11</sup>-), (-O-), (>C=O) or (-S-), and in which groups  $R^{11}$  and  $R^{15}$  are substituent groups as defined above.

39. A detergent composition according to claim 38, in which the enhancing agent is described by the general formula II:

in which the substituent groups R<sup>1</sup>-R<sup>8</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and

esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted or substituted or substituted or phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>;

which substituent group R9 represents any of the following radicals: hydroxy, formyl, carboxy and esters and 15 salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1-C_3$ -20 alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1 - C_5$ -alkyl, and  $C_1 - C_5$ -alkoxy groups may furthermore be 25 saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups  $R^1$ - $R^8$  may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-), (-CH=N-),  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n represents an integer of from 1 to 3,  $R^9$  is a substituent group as defined above and  $R^{10}$  is defined as  $R^9$ .

40. A detergent composition according to either of claims 37-38, in which the enhancing agent is described by the following formula:

in which formula E represents a single bond, a carbonyl 5 group or one of the following groups:  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or (-NR<sup>11</sup>-), in which n represents an integer from 1 to 2. The substituents groups R1-R11 may be identical or different, independently being one of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, car-10 bamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl,  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl,  $aryl-C_1-C_5-alkyl;$  which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice 15 with a substituent group R14; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ alkyl, and  $aryl-C_1-C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be 20 unsubstituted or substituted with one or more substituent groups R14;

which substituent group R<sup>14</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonoxy amd esters and

salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which specific formula two of the substituent 15 groups  $R^5$  and  $R^6$  may together form a group -B-, in which B represents the groups:  $(-CH=N-)_n$ , (-CH=CH-) or  $(-CHR^{14}-)$  in which groups n represents an integer of from 1 to 2 and  $R^{14}$  is a substituent group as defined above.

- 41. A detergent composition according to claim 40, 20 in which the enhancing agent is 4-amino-4'-methoxystilbene, 4,4'-diaminostilbene-2,2'-disulfonic acid, iminostilbene, 4,4'-dihydroxybenzophenone, N-benzylidene-4-biphenylamine, 4,4'-diaminodiphenylamine, 4,4'-dimethoxy-N-methyldiphenylamine, 2,7-diaminofluorene or triphenylamine.
- 25 42. A detergent composition according to either of claims 37-38, in which the enhancing agent is described by the following formula:

in which formula X represents one of the following groups: (-O-), (-S-),  $(-NR^{15}-)$ ,  $(-CHR^{15}-)$ , (>C=O), (-CH=CH-), (-CH=CH-)CH=N-) and the substituent groups  $R^1-R^9$  and  $R^{15}$ , which may be identical or different, independently represents any of the 5 following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C1-C14-alkyl, C1-C5alkoxy, carbonyl-C,-Cs-alkyl, aryl-C,-C5-alkyl; which carbamoyl, 10 sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R10; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{10}$ ; and which  $C_1 - C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl, and aryl- $C_1-C_5$ -alkyl groups 15 may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R10;

which substituent group R<sup>10</sup> represents any of the following radicals: hydroxy, formyl, carboxy and esters and 20 salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be 25 unsubstituted or substituted once or twice with hydroxy,  $C_1 - C_5$ alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; 30 and which  $C_1-C_5$ -alkyl, and  $C_1-C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, 35 and sulfamoyl;

or in which general formula two of the substituent groups  $R^1-R^8$  may together form a group -B-, in which B represents any of the following the groups:  $(-CHR^{10}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or  $(-N=CR^{10}-NR^{11}-)$ , in which groups no represents an integer of from 1 to 3,  $R^{10}$  is a substituent group as defined above and  $R^{11}$  is defined as  $R^{10}$ .

- 43. A detergent composition according to claim 42, in which X in the formula is (-0-) or (-S-) and  $\mathbb{R}^5$  in the formula is an alkyl group which may be further substituted.
- 44. A method according to either of claims 42-43, in which the enhancing agent is 10-methylphenothiazine, 10-phenothiazine-propionic acid, N-hydroxysuccinimide-10-phenothiazine-propionate, 10-ethyl-4-phenothiazine-carboxylic acid, 10-ethylphenothiazine, 10-propylphenothiazine, 10-isopropyl-phenothiazine, methyl-10-phenothiazinepropionate, 10-phenyl-phenothiazine, 10-allylphenothiazine, 10-(3-(4-methyl-1-piperazinyl)propyl)phenothiazine, 10-(2-pyrrolidinoethyl)-phenothiazine, 2-acetyl-10-methylphenothiazine, 10-methyl-phenoxazine, 10-(2-hydroxyethyl)phenothiazine, 10-(2-hydroxyethyl)phenothiazine, 10-(2-hydroxyethyl)phenothiazine.
  - 45. A detergent composition according to either of 37-38, in which the enhancing agent is a biphenyl derivative of the following formula:

in which the substituent groups R<sup>1</sup>-R<sup>10</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>11</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>11</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or

which substituent group  $R^{11}$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and 20 salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_3$ alkyl, C,-C,-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: 25 hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the 30 following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl.

46. A detergent composition according to claim 45, in which the enhancing agent is benzidine, 3,3'-dimethyl-35 benzidine, 3,3'-dimethoxybenzidine, 3,3',5,5'-tetramethyl-

benzidine, 4'-hydroxy-4-biphenylcarboxylic acid, or 4,4'-dihydroxybiphenylene.

47. A detergent composition according to either of claims 37-38, in which the enhancing agent is 2-(p-amino-5 phenyl)-6-methylbenzothiazole-7-sulfonic acid, N-(4-(dimethyl-amino)benzylidene)-p-anisidine, 3-methyl-2-benzothiazolinone(4-(dimethylamino)benzylidene)hydrazone or vanillin azine.

48. A detergent composition according to either of claims 37 and 39, in which the enhancing agent is a naphthalene 10 derivative of the following formula:

in which the substituent groups R<sup>1</sup>-R<sup>8</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>9</sup>; and which one or more substituent groups R<sup>9</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>9</sup>;

which substituent group  $R^9$  represents any of the following radicals: hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof,

sulfamoyl, phospho, phosphono, phosphonooxy amd esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-salkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

- or in which general formula two of the substituent groups  $R^1-R^8$  may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>,  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n represents an integer of from 1 to 3,  $R^9$  is a substituent group as 20 defined above and  $R^{10}$  is defined as  $R^9$ .
- 49. A detergent composition according to claim 48, in which the enhancing agent is 6-hydroxy-2-naphtoic acid, 7-methoxy-2-naphtol, 7-amino-2-naphthalene sulfonic acid, 5-amino-2-naphthalene sulfonic acid, 1,5-diaminonaphthalene, 7-25 hydroxy-1,2-naphthimidazole, 5-amino-2-naphthalenesulfonic acid, or 7-methoxy-2-naphtol.
  - 50. A detergent composition according to any of claims 37-49, which further comprises one or more other enzymes, in particular a protease, a lipase, an amylase, a cellulase, and/or an peroxidase.

I HO COOH

HO COOH

$$HO$$
 $HO$ 
 $HO$ 

Fig. 1A

VIII

$$CH_3$$

VIII

 $R_2$ 
 $R_3$ 
 $R_4$ 
 $R_4$ 
 $R_5$ 
 $R$ 

но — — — соон **Fig. 1B** 

XIV

Fig. 1C

ADDITION , MONOSANI (MAN)

XXII 
$$C_{2}H_{5}$$
  $C_{2}H_{5}$   $C_{2}H_{5}$ 

Fig. 1D

XXXIII

$$H_3C$$
 $H_3C$ 
 $H_3C$ 

Fig. 1E

Fig. 1F

A 605 nm

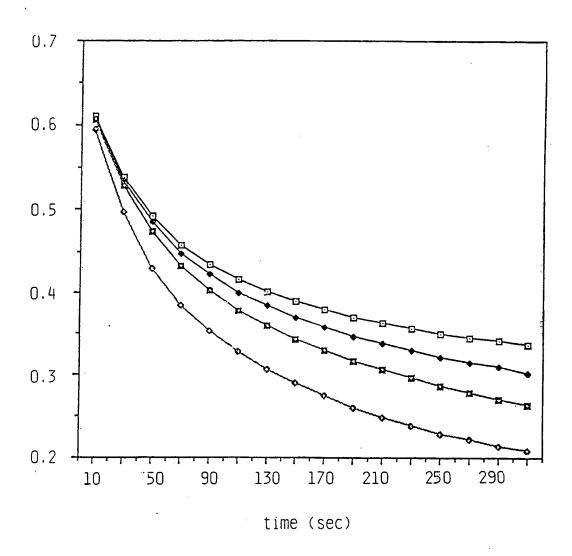


Fig. 2

#### A. CLASSIFICATION OF SUBJECT MATTER IPC5: C12N 9/02, D21C 9/10, C11D 3/386 According to International Patent Classification (IPC) or to both national classification and IPC **B. FIELDS SEARCHED** Minimum documentation searched (classification system followed by classification symbols) IPC5: C12N, C11D, D21C Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched SE,DK,FI,NO classes as above Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CA, WPI, IFIPAT, BIOSIS C. DOCUMENTS CONSIDERED TO BE RELEVANT Relevant to claim No. Citation of document, with indication, where appropriate, of the relevant passages Category\* 1-50 WO, A1, 9105839 (NOVO NORDISK A/S ET AL), 2 May 1991 (02.05.91), see claims WO, A1, 9220857 (HANS-PETER HEINSBERGER), 26 November 1992 (26.11.92) 1-50 Α See patent family annex. Further documents are listed in the continuation of Box C. later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive "E" erlier document but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) step when the document is taken alone document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination document referring to an oral disclosure, use, exhibition or other being obvious to a person skilled in the art document published prior to the international filing date but later than "&" document member of the same patent family the priority date claimed Date of mailing of the international search report Date of the actual completion of the international search 1 1 -10- 1994 29 Sept 1994 Authorized officer Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Carolina Gomez Lagerlöf Telephone No. +46 8 782 25 00 Facsimile No. +46 8 666 02 86

Form PCT/ISA/210 (second sheet) (July 1992)

## INTERNATIONAL SEARCH REPORT Information on patent family members

27/08/94

nternational application No.
PCT/DK 94/00210

Patent document cited in search report		Publication date		nt family mber(s)	Publication date	
WO-A1-	9105839	02/05/91	AT-T- AU-B- AU-A- CA-A- CN-A- DE-D- EP-A,B- SE-T3- EP-A,B- JP-T- JP-T- US-A- WO-A-	108484 646645 6515790 6516090 2067748 1051600 69010691 0495836 0495836 0497794 5500899 5503542 5273896 9105858	15/07/94 03/03/94 16/05/91 16/05/91 14/04/91 22/05/91 00/00/00 29/07/92 12/08/92 25/02/93 10/06/93 28/12/93 02/05/91	
WO-A1-	9220857	26/11/92	AU-A- CA-A- CN-A- DE-A- EP-A- FI-D- NO-D-	1793392 2103260 1068161 4137761 0584176 935067 934148	30/12/92 18/11/92 20/01/93 19/11/92 02/03/94 00/00/00 00/00/00	

THIS PAGE BLANK (USPTO)